METHOD, ~~BAG AND DISPOSABLE SET FOR RECIRCULATION WASHING OF BLOOD CELLS~~

BACKGROUND OF THE INVENTION

This invention relates to recirculation washing of blood cells using a spinning membrane filter, and in particular to recirculation washing of blood cells in a magnetic cell selection apparatus.

Fischel U.S. patent 5,034,135, issued Jul.23, 1991, and Schoendorfer U.S. patent 5,053,121 ~~5,035,121~~, issued Oct. 1, 1991 disclose spinning membrane filters comprising a cylindrical housing and concentric grooved cylindrical rotor. The rotor is covered with a membrane the membrane is spaced from the inner wall of the housing. Blood is introduced into the gap between the membrane and housing. Filtrate passes through the membrane, into the grooves of the rotor, into tubes which communicate with the grooves, and out the bottom center of the spinning membrane filter. Concentrated cells are removed from the gap. Figs. 7 and 8 in the Fischel patent illustrate a cell washing modification in which a porous wall is interposed between the membrane and the inner wall of the housing. Blood is introduced into the gap between the membrane and the porous wall and an isotonic wash solution is introduced into the gap between the porous wall and the inner wall of the housing. Fig. 6 in the Schoendorfer patent illustratse introduction of a rinse solution with the blood. Schoendorfer et al. U.S. patent 5,053,121 ~~5,035,121,~~ issued Oct. 1, 1991, discloses use of two spinning membrane filters in series or parallel. A washing solution is introduced into at least one of the spinning membrane filters.

Duff U.S. patent 5,234,608, issued Aug. 10, 1993, discloses a spinning membrane filter of the type which is preferred for use in conjunction with this invention. According to the disclosure, cell-rich concentrate is removed from the upper portion of the gap between the membrane and the inner wall of the housing, cell-poor plasma filtrate is removed from the bottom center of the spinning membrane filter. Source cell suspension is mixed with cell-rich concentrate and introduced to the lower portion of the gap area.

Schoendorfer et al. U.S. patents 4,675,106, issued Jun. 23, 1987, 4,753,729, issued Jun. 28, 1988, and 4,816,151, issued Mar. 28, 1989, disclose drive mechanisms for spinning membrane filters.

Moubayed et al. U.S. patent 5,536,475 discloses a semi-automated instrument for selection of blood cells using paramagnetic beads which are coated with a binding agent such as an antibody which binds specifically to the cells to be selected. The instrument comprises a primary magnet associated with a primary container and a secondary magnet associated with a secondary container. Blood cells, liquid and beads are agitated in the primary container to form a conjugate between the beads and the selected cells. The primary magnet is then moved into a position adjacent the primary container to magnetically capture the bead/cell conjugate and the non-selected cells and liquid are removed. The primary magnet is then moved into a position away from the primary container to release the bead/cell conjugate. Wash solution is added and the contents of the primary container are agitated, then the primary magnet is moved into the position adjacent the primary container to again capture the bead/cell conjugate and the wash solution is removed. The primary magnet is again moved into a position away from the primary container to release the bead/cell conjugate. Liquid containing a reagent which releases the selected cells from the beads is added and the contents are again agitated. The primary magnet is again moved into the position adjacent the primary container to capture the beads. The released cells and liquid are introduced to the secondary container. The secondary container is positioned adjacent to the secondary magnet to capture any beads which may have escaped the primary magnet. The instrument is used with a disposable set comprising plastic bags for wash liquid, cell suspension and bead suspension, interconnected with plastic tubing.

The semi-automated instrument disclosed in the Moubayed et al. patent is sold by Baxter Healthcare Corporation. under the trademark Isolex® 300 SA. A modified version of the instrument is sold by the Baxter Healthcare Corporation under the trademark Isolex® 300i. The 300i differs from the 300 SA in that it is fully automated and it includes a spinning membrane filter for washing the selected cells and also for removing platelets from the source cells prior to

selection.

Chapman et al. International Publication WO 95/13837, published 26 May 1995, discloses a peristaltic pumping assembly of a type which is used to move fluids in the Isolex® 300 SA and Isolex® 300i instruments. Deniega et al. International Publication WO 95/141~~4~~72, published 26 May 1995, discloses an organizer frame of a type which is used with the peristaltic pumping assembly in the Isolex® 300 SA and Isolex® 300i instruments. The organizer frame is also used on a machine for separation of platelets from whole blood. Deniaga discloses a tubing set which includes a spinning membrane filter and a reservoir for platelet-poor packed blood cells. The reservoir has a top and bottom port. Packed cells from the outlet of the spinning membrane filter enter through the top inlet port of the reservoir. Whole blood from a patient enters through the bottom inlet port.

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Recirculation washing of selected blood cells is performed in the Isolex® 300i utilizing the spinning membrane filter in conjunction with a recirculation wash bag which has both inlet and outlet ports at the bottom and no port at the top. The bag is a 600 ml bag with the inlet and outlet ports separated by about 2 inches. The bag has been able to concentrate cell suspensions that normally start at about 400 ml. This bag performed better when it was occasionally massaged. This is the only way to process more than about 5×10^{10} cells in the bag.

The above-cited U.S. patents and International Publications are each incorporated herein by reference.

SUMMARY OF THE INVENTION

This invention includes a method, a bag and a disposable set for recirculation washing of blood cells. The invention can be used for washing of blood cells in a magnetic cell selection instrument, but can also be used for washing whole blood or other blood cell products.

The recirculation wash bag is a flexible plastic bag which has a top port and a bottom

port. In one embodiment, an integral coarse filter comprising a tube of semi-rigid plastic mesh extends from the top port into the bag. This filter provides mild resistance to larger cell aggregates. In another embodiment, the bag includes a bubble trap at the top comprising tubing extending into the bag from the top port. In the preferred embodiment, the bag includes both the semi-rigid integral filter and the bubble trap; the tubing for the bubble trap fits inside the plastic mesh tube to provide a space to accumulate air around the tubing. When a system incorporating the bag is primed with buffer solution, vacuum is pulled on the bag. Because the filter is semi-rigid, it holds open a path through the otherwise collapsed bag for the cells to move up to the top port.

The method of the invention utilizes a flexible plastic recirculation wash bag and a spinning membrane filter. The spinning membrane filter has an inlet port for a diluted suspension of blood cells in buffer solution, a first outlet port for filtrate, and a second outlet port for a concentrated suspension of blood cells in buffer solution. The recirculation wash bag has a top outlet port and a bottom inlet port. Preferably, the recirculation wash bag includes the integral coarse filter and bubble trap described above.

The method comprises withdrawing a suspension of blood cells in buffer solution from the recirculation wash bag through the top port, mixing the suspension with additional buffer solution to form a diluted suspension of blood cells in buffer solution, feeding the diluted suspension into the spinning membrane filter through the inlet port, withdrawing filtrate comprising buffer solution from the spinning membrane filter through the first outlet port, withdrawing a concentrated suspension of blood cells in buffer solution from the spinning membrane filter through the second outlet port, feeding the concentrated suspension into the bag through the bottom port, and continuing the recirculation washing until the desired amount of washing has been achieved. A method for determining when the desired amount of washing has been achieved, based on an estimate of "residual," is described below. The residual represents the target component for reduction (e.g., platelets, antibody, etc., as described below).

In one embodiment of the method, the suspension of blood cells withdrawn through the top port of the recirculation wash bag is mixed with unwashed blood cells as well as buffer solution before feeding the diluted suspension into the spinning membrane filter. In one aspect of this embodiment, the unwashed blood cells include platelets, the filtrate comprises a suspension of platelets in buffer solution, and the recirculation washing is continued until the platelet content of the concentrated suspension of cells has been reduced to the desired level.

In another embodiment of the method, the recirculation wash bag at the beginning of the recirculation wash procedure contains, in addition to blood cells, an antibody which specifically binds an antigen on certain of the blood cells, the filtrate comprises a suspension of the antibody in the buffer solution, and the recirculation washing continues until the concentrated suspension of cells contains the desired amount of excess, unbound antibody.

In another embodiment of the method, the recirculation wash bag at the beginning of the recirculation wash procedure contains blood cells which have been selected in a magnetic cell selection procedure and a peptide release agent which was used to release the selected cells from a cell/magnetic bead conjugate, the filtrate comprises a solution of the peptide release agent in buffer solution, and the recirculation washing is continued until the peptide release content of the concentrated suspension of cells has been reduced to the desired level.

The disposable set of the invention comprises the recirculation wash bag and the spinning membrane filter having ports as described above, and a filtrate bag, plus associated tubing, including tubing for a buffer solution bag. Plastic tubing connects the top port of the recirculation wash bag to a mixing zone. Plastic tubing with a buffer bag spike coupler at one end is connected to the same mixing zone. The mixing zone is connected by plastic tubing to the inlet port of the spinning membrane filter. The first outlet port of the spinning membrane filter is connected by plastic tubing to the inlet port of the filtrate bag. The second outlet port of the spinning membrane filter is connected by plastic tubing to the bottom port of the recirculation wash bag.

The disposable set may also include other bags and associated tubing for use in a magnetic cell selection instrument, such as a bag for antibody suspension in buffer solution, a bag for peptide release agent solution in buffer solution, a bag for a suspension of the non-selected cells in buffer solution, and an end product bag for washed cells. A bag for unwashed cells (also referred to as a cell source bag) and/or a bag for buffer solution may be included in the set, but in the preferred embodiment these items are supplied separately.

Use of a flexible recirculation wash bag with ports at the top and bottom and flow from bottom to top provides several advantages as compared to a bag with inlet and outlet ports at the bottom, as currently used on the Isolex® 300i. First, using a flexible bag allows the volume to be varied depending on the number of cells. Exiting from the top has the advantage of removing the less dense supernatant preferentially. This aids in making the concentration ratio high. (The importance of high concentration ratio is discussed below). For large volumes or slow flow rates, some sedimentation of the larger cells also aids in reducing the cell concentration at the outlet port. The system has the advantage of having the most washed and most concentrated cells at the bottom with the least washed and least concentrated cells at the top. Additional advantages include the following: (1) allows accurate residual estimates which in turn allow optimal residual levels instead of just reduction; (2) provides more uniform processing of cells which leads to a more uniform product for the selection process; (3) manual massaging of the bag during the wash is not required, permitting hands-free operation.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the preferred embodiment of the recirculation wash bag of this invention. In the description which follows the recirculation wash bag having the configuration shown in Fig. 1 is referred to as the IsoFlow™ bag.

Fig. 2 illustrates a disposable set of this invention which is adapted for use on a magnetic cell selection device such as the Isolex® 300i.

Fig. 3 illustrates a disposable cell wash set of the invention which is adapted for use on a stand-alone cell washing apparatus.

DETAILED DESCRIPTION OF THE INVENTION

IsoFlow™ recirculation wash bag

Referring to Fig. 1, the IsoFlow™ bag is indicated generally by the numeral **5**. The bag is made of a flexible plastic such as and includes bottom port **1** and top port **2**. An integral coarse filter comprising a tube of semi-rigid plastic mesh **3** extends from the top port into the bag to within about 1/2 to 3 inches, preferably about 1 inch, from the bottom of the bag. The mesh tube is about 1/2 to about 1.5 inches in diameter, preferably about 1 inch in diameter, and is preferably closed at its lower end. The tube's mesh (opening) size is in the range of about 80-400 microns, preferably about 230 microns. The bag includes a bubble trap at the top which is created by inserting tubing **4** into the top port about 1/2 to 3 inches, preferably about 1.5 inches. Suitable materials of construction include polyvinyl chloride (PVC) for the bag, polyester (e.g. Cleartuf®, shell) for the mesh tube filter, and PVC for the tubing. Volume of the bag can vary, but will generally be between 100 and 1500 ml. As presently designed for use on the Isolex® 300i, the bag holds a volume of 400 ml. The mesh could be replaced by some other semi-rigid, rigid or combination structure that facilitates flow from bottom to top.

Isolex® 300i cell washing system

Referring to Fig.2, the disposable set of this invention comprises the IsoFlow™ bag **5** and spinning membrane filter **6** and associated tubing, including tubing for connecting a bag containing buffer solution. Spinning membrane filter **6** (sometimes referred to simply as "spinning membrane" or "spinner") has the construction shown in Fig. 2 of Duff U.S. patent 5,234,608. The membrane is a nominal 4 micron polycarbonate membrane. The buffer solution bag is not shown, but is indicated at **7**; it is a standard flexible plastic bag with a bottom outlet port, and is supplied separately. The top port **2** of IsoFlow™ bag **5** is connected by tubing **8** having a sampling device **8a** to the bottom right channel **9b** (indicated by dotted lines) of clamp manifold **9**. Channel **9b** is a mixing zone for mixing cells from IsoFlow™ bag **5** with buffer

solution from **bag** 7 and (in the platelet separation step described below) with unwashed cells from bag **44**. Channel **9b** of clamp manifold **9** is connected by tubing **10** to the inlet port **11** of spinning membrane filter **6**. The bottom port 1 of IsoFlow™ bag **5** is connected by tubing **12** to the bottom left channel of clamp manifold **9** and tubing **13** connects the bottom left channel of clamp manifold **9** to the outlet port **14** of spinning membrane filter **6**. Tubing **15** connects the outlet port of buffer solution **bag 7** to the top right channel of clamp manifold **16**; tubing **17** connects the top right channel of clamp manifold **16** to the bottom left channel of clamp manifold **18**; tubing **19** connects the bottom left channel of clamp manifold **18** to the bottom right channel of clamp manifold **18** and tubing **20** connects the bottom right channel of clamp manifold **18** to the bottom right channel **9b** of clamp manifold **9**. Tubing **15** is connected to a buffer bag spike coupler **21** and a sterilizing filter **22**. Tubing **23** connects filtrate outlet port **24** of spinning membrane filter **6** with the top right channel of clamp manifold **25**. Tubing **26** connects the top right channel of clamp manifold **25** with Y-connector **27**. Tubing **28** connects Y-connector **27** to the inlet port **29** of filtrate (waste) bag **30**. On tubing **28** is a clamp **31**. Tubing **32** connects Y-connector **27** to Y-connector **33.** Tubing **32** carries a clamp **40**. Tubing **34** connects Y-connector **33** to inlet port **35** of waste bag **36**. Tubing **37** connectes Y-connector **33** to inlet port **38** of waste bag **39**. Tubing **41** connects the top right channel of clamp manifold **25** to pressure transducer protector **42**.

There are three configurations of clamp manifolds shown in Fig. 2. All configurations have clamps capable of obstructing the tubing that runs through them on a flat platen (not shown) in the center of the manifolds. The dotted lines in the upper and/or lower portions of the clamp manifolds indicate the locations of channels within the manifolds. The dotted lines in clamp manifold **45** show that the bottom channel connects all 4 tubes. The dotted lines in clamp manifolds **9** and **18** show that there are two bottom channels--the left channel connects the two left tubes and the right bottom connects the two right tubes. The dotted lines in clamp manifolds **16** and **25** show that the bottom left channel connects the tubes on the left and the top right channel connects the tubes on the right.

In the preferred embodiment illustrated in Fig. 2, the disposable set of the invention also includes other bags and containers and associated tubing adapted for use on a magnetic cell separation instrument such as the the Isolex® 300i. Tubing **43** connects a cell source bag (not shown, but indicated at **44**) with the bottom channel of clamp manifold **45**. Tubing **46** connects the bottom channel of clamp manifold **45** with the bottom left channel **18a** of clamp manifold **18**. Channel **18a** is a mixing zone for buffer from **bag 7** and unwashed cells from bag **44**. Tubing **43** is connected to a starting cells spike coupler **47**.

Bag **48** is a bag for antibody which reacts specifically with cells to be selected on the Isolex® 300i. For example, where CD34+ cells are to be selected, bag **48** will contain anti-CD34 antibody. The bag has an injection site **49** for injection of the antibody solution and an outlet port **50** connected to a sterilizing filter **51**. Tubing **52** connects sterilizing filter **51** to the bottom channel of clamp manifold **45**.

Bag **53** is a bag for a peptide release agent which displaces the antibody from the cells after the cells have been magnetically selected. Bag **53** has an injection site **54a** for injection of a solution of the peptide and an outlet port **54** connected to a sterilizing filter **55**. Tubing **56** connects sterilizing filter **55** to the bottom channel of clamp manifold **45**.

Cylinder **57** is the primary magnet separation chamber. It has a vent filter **59** and an injection site **58** for injection of paramagnetic microbeads coated with an antibody which binds specifically to the antibody in bag **48**. It has a bottom port **60** which serves as both inlet and outlet for cell suspensions. In use it is mounted on a rocker mechanism as described in Moubayed et al. U.S. patent 5,536,475. Port **60** is connected by tubing **61** to the bottom left channel of clamp manifold **16**. That channel is connected by tubing **62** to the right top channel of clamp manifold **16**. The top right channel of manifold **16** is connected by tubing **72** to the top right chamber of clamp manifold **25**. The bottom left channel of clamp manifold **16** is also connected by tubing **63** to Y-connector **64** and the latter is connected by tubing **65** to the bottom channel of clamp manifold **45**. Y-connector **64** is also connected by tubing **66** to a pressure

transducer protector **67**.

Bag **68** is the secondary magnet separation bag described in Moubayed et al. U.S. patent 5,536,475. It has inlet port **69** and outlet port **70**. Inlet port **69** is connected by tubing **71** to the bottom left channel of clamp manifold **18**. Outlet port **70** is connected by tubing **73** to the bottom right channel of clamp manifold **18**.

Bag **74** is a selected cell wash bag. It has two bottom ports. Inlet port **75** is connected by tubing **77** which has a sampling device **77a** to the bottom right channel **9b** of clamp manifold **9**. Outlet port **76** is connected by tubing **78** to the bottom left channel of clamp manifold **9**. If desired, an IsoFlow™ bag can be substituted for the selected cell wash bag.

Bag **79** is an end product bag. It has an injection site **80** and an inlet port **81**. Tubing **82** carrying sampling device **82a** and clamp **83** connects inlet port **81** with the bottom channel of clamp manifold **45**.

Frame **84** is an organizer frame as described in Denieaga et al. International Publication WO 95/14142 for use with a peristaltic pump assembly (not shown) as described in Chapman et al. International Publication WO 95/13837. Tubing **13**, **15**, **26** and **46** each passes through one of the four pumping modules of the peristaltic pump assembly.

The volume of bags can vary, depending upon the volume of cells to be processed. In the the commercial Isolex® 300i instrument, each of bags **30**, **36** and **39** has a volume of 2000 ml, each of bags **48**, **53** and **79** has a volume of 150 ml, and bag **74** has a volume of 600 ml. For use in this system, the IsoFlow™ bag **5** has a volume of 400 ml.

At the beginning of a cell selection procedure, the disposable set of Fig. 2 is placed on the Isolex 300i. **Bag** 7 containing buffer and bag **44** containing source cells are attached. The source cells are typically a leukapheresis product from a cell separation device such as a Fenwall

3000 CS. The buffer bag has a capacity of 4000 ml and a starting volume of at least 3500 ml. The cell source bag has a capacity of 1000 ml and a starting volume of about 500 ml. By appropriate operation of clamps in the clamp manifolds and the pumps on tubing **13**, **15**, and **46**, buffer solution is added to the following elemments and connecting tubing to prime the system: Isoflow™ bag **5**, secondary magnet pouch **68**, spinning membrane filter **6**, filtrate bag **30**, selected cell wash bag **74**, release agent bag **53**, antibody bag **48**, cell source bag **44**. During the prime, fluid is added to the Isoflow™ bag, the air is removed from the top part of the bag, more fluid is added through the bottom part, and excess air is released through tubing **8** to waste bag **30**.

At this point the system is ready for removal of platelets from the leukapheresis product in cell source bag 44, using the method of this invention. For purpose of the following description: clamps in clamp manifold 45 are designated clamps C1, C2, C3, C4; clamps in clamp manifold 9 are designated C5, C6,C7, C8; clamps in clamp manifold 16 are designated C9, C10, C11, C12; clamps in clamp manifold 18 are designated C13, C14, C15, C16; clamps in clamp manifold 25 are designated C17, C18, C19, C20; the pump on tubing 46 is designated P1, the cell source pump; the pump on tubing 15 is designated P2, the buffer pump; the pump on tubing 13 is designated P3, the recirculation pump; the pump on line 26 is designated P4, the filtrate pump; and the rotor of spinning membrane filter **6** is designated as pump P5.

Prior to beginning cell wash, clamps C6, C8, C10, C11, C12, C14, C16 and C20 are opened, pumps P2, P3, P4 and P5 are moving. This circulates buffer solution from **bag 7**, into the inlet port **11** and out of outlet ports **14** and **24** of spinning membrane filter **6**, into bottom port **1** and out of top port **2** of IsoFlow™ bag **5**, and into filtrate bag **30**.

To conduct recirculation washing of the blood cells for platelet removal, clamps C1, C6, C8, C12, C14, C16 and C20 are open, pumps P1, P2, P3, P4, and P5 are moving. A suspension of unwashed blood cells is withdrawn from cell source bag **44** through tubing **43** to the bottom channel of clamp manifold **45**, then out through tubing **46** to the bottom left channel **18a** of

clamp manifold **18** where it is mixed with buffer solution. The buffer solution is withdrawn from buffer **bag 7** through tubing **15** to the top right channel of clamp manifold **16**, then out through tubing **17** to the bottom left channel **18a** of clamp manifold **18**. The diluted suspension of blood cells in buffer solution flows out of the bottom left channel **18a** through tubing **19** into the bottom right channel of clamp manifold **18**, then out through tubing **20** to the bottom right channel **9b** of clamp manifold **9**, where it is mixed with additional buffer solution from top port **2** of Isoflow™ bag **5**. The diluted suspension of blood cells in buffer solution flows from channel **9b** through tubing **10** to the inlet ~~inle~~ port **11** of spinning membrane filter **6**. Platelets, a few red cells, and buffer flow through the membrane and out through outlet port **24** through tubing **23** to the top right channel of clamp manifold **25**, then out through tubing **26** and **28** to filtrate bag **30** (clamp **31** open, clamp **40** closed). (The nominal 4 micron ~~miron~~ membrane used removes about 95% of platelets from a leukapheresis product, while about 50% of red cells are also removed.) A concentrated suspension of blood cells in buffer flows from the exit port **14** of spinning membrane filter **6** through tubing **13** to the bottom left channel of clamp manifold **9**, then out through tubing **12** through the bottom port **1** into Isoflow™ bag **5**. As the process continues, a suspension of blood cells in buffer solution flows out of the top of the Isoflow™ bag **5**. These cells are mixed in mixing zone **9b** with unwashed cells from source bag **44** and are recirculated through the spinning membrane filter **6**. Recirculation washing is continued until the desired level of platelet removal has been achieved.

After platelet removal, antibody in buffer solution is transferred to the concentrated suspension of blood cells in buffer solution in the Isoflow™ bag **5**. For transfer of antibody solution from bag **48** to Isoflow™ bag **5**, clamps C3, C6, C8, C14, C16 and C20 are open and pumps P1, P3 and P5 are moving. The antibody and cells are mixed in mixing zone **9b**. Then the antibody tubing is rinsed with buffer solution while the antibody/cell suspension circulates through the Isoflow™ bag **5** and spinning membrane filter **6**. This occurs with clamps C6, C8, C10, C11, C14, C16 and C20 open,and with pumps P1, P2, P3 and P5 moving. Next the antibody/cell suspension is circulated through the Isoflow™ bag **5** and spinning membrane filter **6** to sensitize the cells by binding with the antibody. This is accomplished with clamps C6, C8

and C20 open, and with pumps P3 and P5 moving.

After the cells have been sensitized by binding with antibody, they are washed to remove excess unbound antibody using the method of this invention. With clamps C6, C8, C12, C14, C16 and C20 open and with pumps P2, P3, P4 and P5 moving, a suspension of blood cells in buffer solution and containing excess unbound antibody is withdrawn from Isoflow™ bag **5** through top port **2** and flows through tubing **8** to the mixing zone **9b** in clamp manifold **9**. Buffer solution is withdrawn from buffer **bag 7** through tubing **15**, clamp manifold **16**, tubing **17**, clamp manifold **18** (left channel), tubing **19**, clamp manifold **18** (right channel) and tubing **20**, as previously described, to mixing zone **9b**, where it is mixed with the suspension of blood cells from Isoflow™ bag **5** to form a diluted suspension of blood cells containing excess unbound antibody. This diluted suspension flows through tubing **10** to inlet port **11** of the spinning membrane filter **6**. Filtrate comprising antibody in buffer solution flows out of outlet port **24**, through tubing **23**, clamp manifold **25**, tubing **26**, tubing **28**, and port **29** into filtrate bag **30**. A concentrated suspension of blood cells in buffer solution flows from the outlet port **14** of the spinning membrane filter **6**, through tubing **13**, clamp manifold **9** (bottom left channel), tubing **12** and bottom port **1** into Isoflow™ bag **5**. The recirculation washing is continued until the cell suspension contains the desired level of unbound antibody.

After antibody sensitization and removal of excess unbound antibody, the cells are transferred to primary magnet separation chamber **57**. Antibody-coated paramagnetic microbeads are mixed with the cells to form a conjugate between the microbeads and the sensitized cells, the conjugate is magnetically separated from the non-sensitized cells, the non-sensitized cells are transferred to waste bag **36**, peptide release agent from bag **53** is added to the chamber **57** to release the selected cells, the selected cells are transferred to the secondary magnet separation bag where any remaining ~~remining~~ microbeads are separated magnetically, and the selected cells are transferred to selected cell wash bag **74**. The selected cells are then recirculation washed to remove excess peptide release agent using spinning membrane filter **6**, all in conventional manner. If desired, selected cell wash bag can be an Isoflow™ bag, and the recirculation wash to

remove peptide release agent can be conducted using the method of this invention. After removal of peptide release agent, the selected cells are transferred to end product bag **79**.

Stand-alone cell washing system

Fig. 3 illustrates a disposable set of the invention which is adapted for use on a stand-alone cell washing apparatus, i.e., an apparatus which does not include a cell selection function such as the magnetic cell selection of the Isolex® 300i instrument.

The disposable set includes Isoflow™ bag **5** having top port **2** and bottom port **1**, spinning membrane filter **6** having inlet port **11** for a diluted suspension of blood cells, outlet port **14** for a concentrated suspension of blood cells, and outlet port **24** for filtrate, and filtrate bag **30** having inlet port **29**. It may also include one or more of washed cell bag **79** having outlet port **81**, unwashed cell bag **44** having outlet port **47**, and buffer solution **bag 7** having outlet port **21**. Top port **2** of Isoflow™ bag **5** is connected by tubing **8** to connector **89**. Port **21** of buffer **bag 7** is connected by tubing **15** to Y-connector **95** and the latter is connected by tubing **20** carrying clamp **C1** to connector **89**. Port **47** of unwashed cell bag is connected by tubing **43** carrying clamp **C3** to Y-connector **93** and then by tubing **91** to connector **89**. Connector **89** serves as a mixing zone for unwashed cells in buffer solution from bag **44**, recirculating cells in buffer solution from bag **5** and buffer solution from **bag 7**. Connector **89** is connected by tubing **10** to inlet port **11** of spinning membrane filter **6**. Filtrate outlet port **24** of spinner **6** is connected by tubing **23** to Y-connector **94** and by tubing **26** to the inlet port **29** of filtrate bag **30**. Connector **95** is connected by tubing **92** carrying clamp **C2** to connector **94**. Connector **94** is connected by tubing **41** to pressure transducer **90.** Oulet port **14** of spinner **6** is connected by tubing **13** to the bottom port 1 of Isoflow™ bag **5**. Y-connector **93** is connected by tubing **82** carrying clamp **C4** to inlet port **81** of washed cell bag 79.

During recirculation washing, a suspension of blood cells in buffer solution is withdrawn from the Isoflow™ bag **5** through the top port **2** and flows through tubing **8** to mixing zone **89**. Unwashed cells in buffer solution are withdrawn from bag **44** through port **47** and

(with clamp **C3** open and clamp **C4** closed) through tubing **43** to Y-connector **93** and then through tubing **91** to mixing zone **89** by the transfer pump **P2**. Buffer solution is withdrawn from **bag 7** through port **21** and tubing **15** to connector **95** by the buffer pump **P2**. With clamp **C1** open, buffer flows through tubing **20** to mixing zone **89**. A diluted suspension of blood cells in buffer solution flows from mixing zone **89** through tubing **10** to inlet port **11** of spinner **6**. A concentrated suspension of blood cells in buffer solution flows through outlet port **14** of spinner **6** through tubing **13** and inlet port **1** into Isoflow™ bag **5** by recirculation pump **P3**. Filtrate flows through outlet port **24** in spinner **6** and tubing **23** to connector **94** and, with clamp **C2** closed, through tubing **26** and inlet port **29** into filtrate bag **30** by pump **P4**. Recirculation washing is continued until the desired amount of target component has been removed from the blood cells. Clamps **C1**, **C2** and **C3** are then closed, clamp **C4** is opened, and the direction of pump **P1** is reversed, so that the suspension of washed cells flows from bag **5** through tubing **8**, **91** and **82** and port **81** into washed cell bag **79**. The lines, bag and spinner are then rinsed by closing clamps **C1** and **C3**, opening clamps **C4** and **C2**, and pumping buffer with pump **P2** in series with pumps **P1** and **P3** to rinse the spinner, Isoflow™ bag and tubing.

System controls

In carrying out the recirculation washing method of this invention, the filtrate rate *(f)* is typically fixed at about 70 ml/min. During the transfer of cells into the wash circuit, the recirculation rate *(r)* provides the primary pressure regulation (using the concentration ratio CR described below) and varies from 14 to 70 ml/min. During the recirculation phase the recirculation rate ranges from about 24 to 70 ml/min. The buffer solution rate *(b)* ranges from 0 to 70 ml/min. to maintain a minimum scale volume and as a secondary pressure regulation mechanism. The rotor of the spinning membrane filter operates at a maximum of 3700 RPM and a minimum of about 2340 RPM during normal processing.

The Isolex® 300i system is automatically controlled using microprocessors. These microprocessors in-turn control 5 banks of 4 clamps each (clamps C1-C20), 1 bank of pumps (pumps P1-P4), 1 spinner motor drive **P5** (drive for the rotor of spinning membrane filter **6**), and

1 rocker assembly for container **57** with an integral magnet carriage to facilitate separation of magnetic beads (not shown, but described in Moubayed et al. U.S. patent 5,536,475). The system uses feedback from 6 weight scales (not shown), 2 pressure transducers (not shown, but attached to line **66** at **67** and to line **41** at **42**, and 3 sets of fluid and tubing detectors (not shown but attached to lines **61**, **66** and **41**). During the Isolex® 300i procedure the bags **44**, **53**, **48** and **79** are hung on weight scales 1, 2, 3 and 4, respectively. Bags **74** and **5** are hung together on weight scale 5. Buffer bag **7** is hung on weight scale 5. Buffer bag **7** is hung on weight scale 6. Bags **36**, **39** and **30** are not hung on a scale. Weight scale 5 is used to determine the cell product volume in the wash circuit by substracting out the reference weight when the Isoflow™ bag is empty. The weight scales are in the tower of the Isolex® 300i instrument.

The stand-alone cell washing system will also run automatically using microprocessors. These microprocessors in turn control 1 bank of 4 clamps each, 1 bank of 4 pumps and 1 spinner motor drive. The system will require feedback from 4 weight scales, 2 pressure transducers, and 3 sets of fluid and tubing detectors.

The size of the cell mass is minimized by increasing the concentration ratio (CR) as far as possible. CR is the ratio of the rate of unwashed undiluted cell volume coming into the spinning membrane filter to the rate of washed cell volume exiting the spinning membrane filter. In the wash circuit, there are four variables to control CR, the recirculation rate *(r)*, the buffer solution rate *(b)*, the cell source rate *(c)*, and the filtrate rate *(f)*. The relationship is $c + b = r + f$, and CR $= c/r = 1 + (f-b)/r$.

For both the Isolex® 300i and the Stand-alone system, the cells are concentrated and washed automatically. We have found that by concentrating, diluting, and concentrating again multiple times, the volume can be more consistently controlled. Thus, between every other cell product cycle through the spinner (i.e., spinning membrane filter) the cell volume is diluted and reconcentrated. If the number of cycles left is predicted to be less than 2.5 cycles, the dilutions stop. During dilutions, the filtrate pump P4 is stopped, the buffer pump P2 runs at a fixed rate

and the recirculation pump P3 runs at about 110% of the buffer rate. This allows the membrane to be rinsed and dilutes the cell concentrate through the port with the more concentrated cells.

The transmembrane pressure is regulated by controlling the concentration ratio CR using the recirculation pump P3. The concentration ratio CR is contolled to a target pressure by a PID (Proportional/Integrative/Derivative) control through the pressure measurements. The pressure measurements are taken from the pressure transducer connected to the filtrate line and are adjusted for the centrifugal effects on the fluid to yield a trans-membrane pressure. If the bag volume drops below the target volume, CR is no longer the controlling parameter. Instead, the scale weight is controlled by the buffer pump P2 and CR is calculated as: CR = c/r. Given CR, the recirculation rate is calculated as r = 70/CR-1 where CR is limited to >=2.

Filtrate rate *(f)* is set to its maximum in order to minimize the time to process the cells. Filtration pressure is an indicator of the concentration of blood cells along the membrane of the spinning membrane filter. However, if either the spinner **6**, buffer pump P2 or recirculation pump P3 are not up to speed, the filtrate rate is reduced. The ratio of the measured spinner **6**, buffer pump, or recirculation pump rate to the respective commanded rate is calculated. The filtrate rate is then calculated as f_1 = 3/4*MRR*TFR + 1/4*TFR, where f_1 is the minimum ratio adjusted rate to be commanded in ml/min, MRR is the minimum rate ratios described above, TFR is the target filtrate rate (70 ml/min). The filtrate rate is further reduced when the pressure error (E_p) described above is less than -5mmHg. When this condition is true the filtrate rate is set to $f_2 = f_1 + E_p + 5$, where f_2 is the final command filtrate rate and f_1 is the minimum ratio adjusted filtrate rate described above. During dilutions, the filtrate rate is set to 0.

Recirculation rate *(r)* is the primary regulating variable. The buffer solution rate *(b)* is used to regulate the concentration ratio CR between values of 1 and 2. The buffer pump P2 provides the primary regulation to the scale weight management control. When the Isoflow™ bag **5** fluid volume weight drops below the target (20-35 ml), the buffer is commanded to about 78 ml/min. This is approximately 8 ml/min faster than the filtrate pump P4. This causes the bag

weight to rise. Once the weight rises about 5 ml, the buffer once again becomes secondary to the concentration ratio control, the buffer pump P2 is regulated according to the equation
$b = (70 + f)/2 - r^*(CR-1)$.

Because the blood cells can be damaged by stress, the ~~contoller~~ controller automatically adjusts the rotor spin rate of the spinning membrane filter. As the recirculation rate *(r)* is decreased the exposure time of the cells in the spinning membrane filter increases as follows:
$t = v/(r+f)$, where t and v are time and volume, respectively, in the spinning membrane. When r slows, stress on the cells increases. The controller counteracts this by decreasing the spin rate linearly when r is reduced.

The amount of washing is based on an estimate of "residual". The residual represents the target component for reduction (e.g., platelets, antibody). This estimate is made possible by the mixing properties of the IsoFlow™ bag. The estimate is calculated similar to how serial dilutions would calculate the residual. However, it is recalculated several times a second. The equation is

$$FSR_i = FSR_{i-1} - (F_i / (B_i + C_i) \times (C_i/V_i) \times FSR_{i-1} \times TA)$$

where i = the discrete time interval

FSR_i = Fraction of Starting Residual at time t_i

FSR_{i-1} = Fraction of Starting Residual at time t_{i-1}

F_i = Filtrate volume moved at rate f measured at time interval i-1 to i in units of ml

B_i = Buffer volume moved at rate b measured at time interval i-1 to i in units of ml

C_i= Cell source moved at rate c measured at time interval i in units of ml, including the rate from the IsoFlow™ bag 5, as well as the rate of addition of unwashed cells, if any, in same units

V_i = cell product volume at time interval i in ml

TA = Target Admittance

The Target Admittance is the unitless constant that represents the ease with which a given substance passes through the membrane (the inverse of membrane impedance). For platelet wash the Target Admittance has been found to be between 0.5 and 1.0 with a preferred setting of 0.7.

For antibody and release agent wash the Target Admittance has been found to be between 0.7 and 1.2 with a preferred setting at 1. The optimal level for the antibody used for $CD34^+$ selection on the Isolex® 300i has been found to be in the range of 50-150 micrograms.

An estimate of the average number of times a cell has been through the spinning membrane acts as a backup for determining when to end a wash. Cell cycles are estimated based on the following equation:

$$\text{Cell cycles}_i = \int (R_j+F_j-B_j)/V_j = \int C_j/V_j$$

where

R_j = Recirculation volume moved at rate *r* measured at time interval j in units of ml, and

Cell cycles$_i$ = Number of cycles through the spinning membrane device that the cell product has experienced at time interval i.